

Supplementary Figures for
SLC15A3-Mediated Dipeptide Metabolism Confers Antimetabolite Resistance in
Lymphoma via mTORC1 Activation

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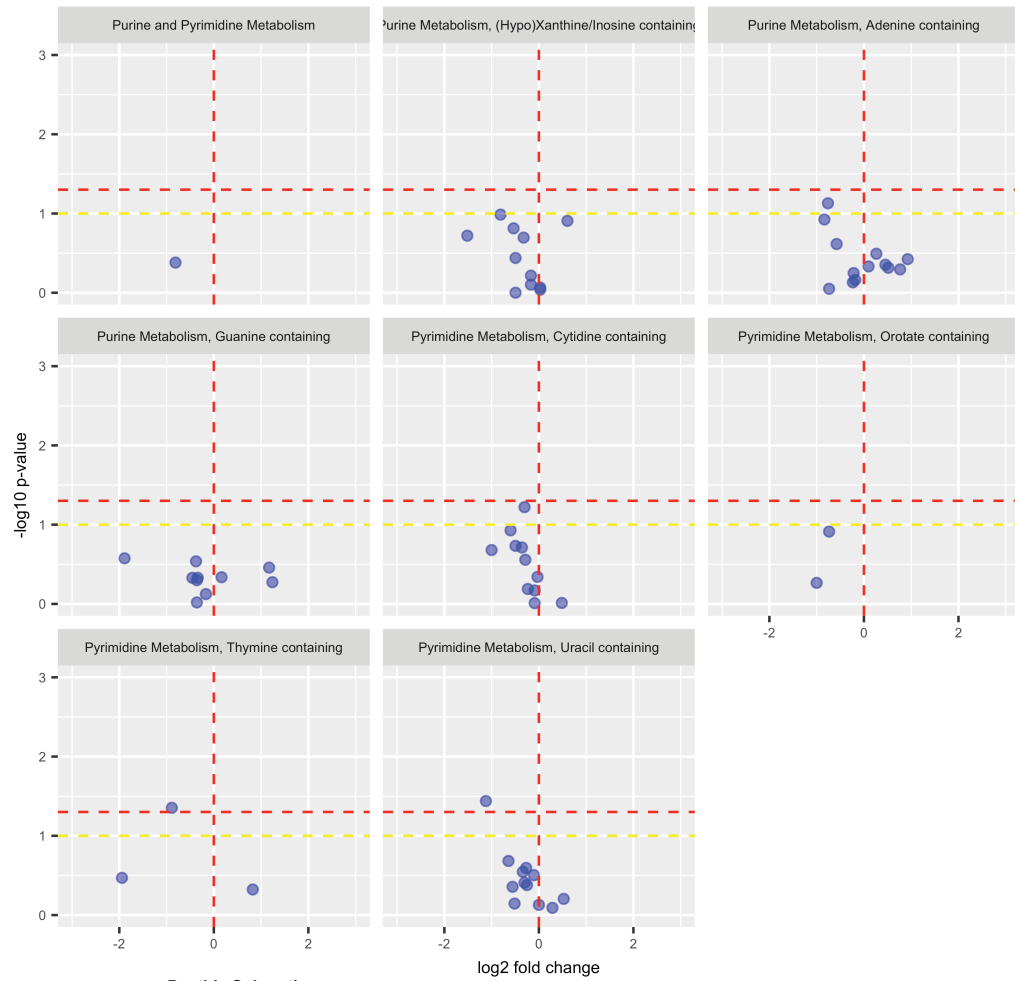
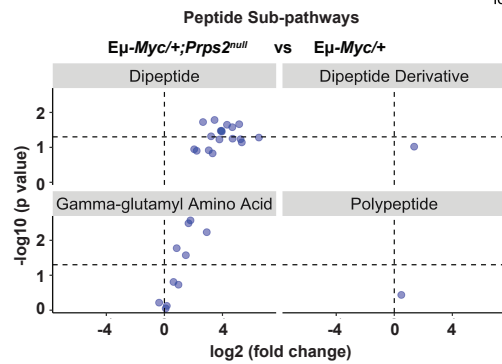
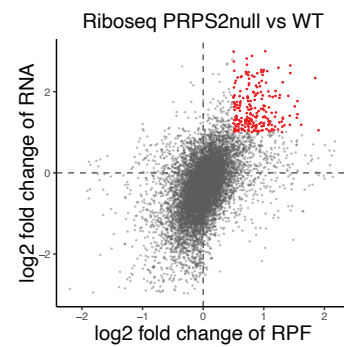
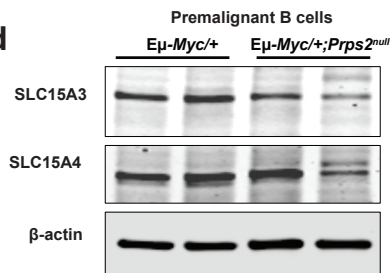
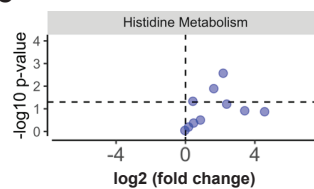
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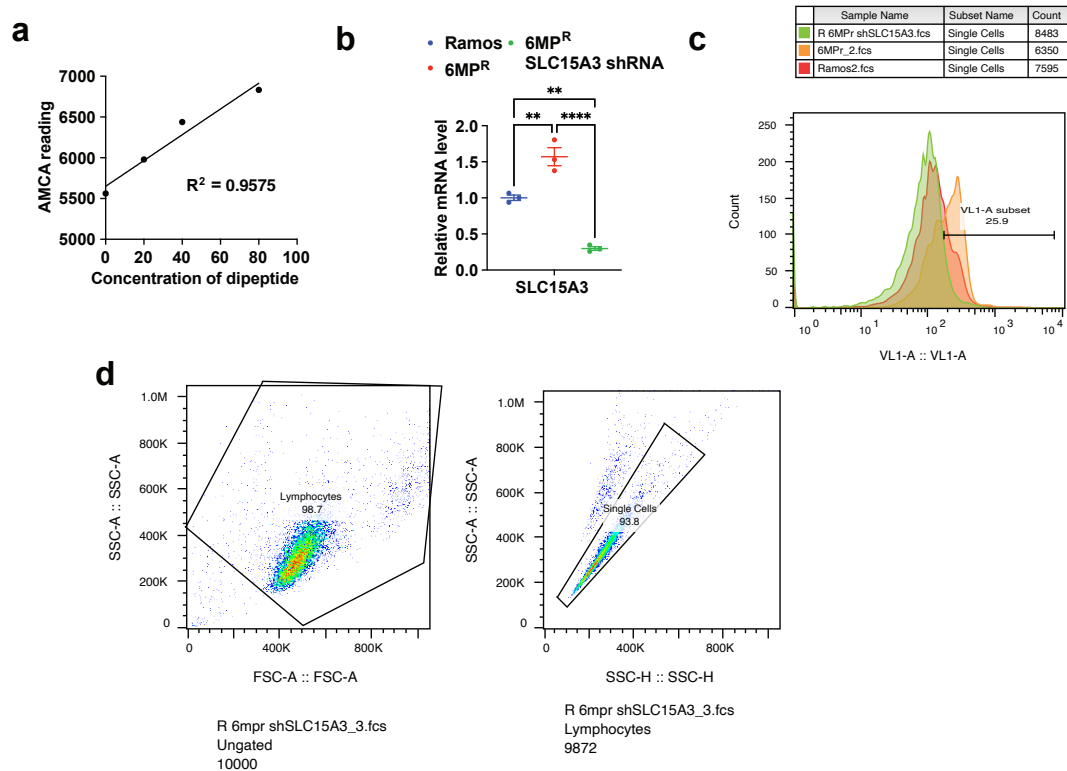
a

Myc KO TD / Myc TD Nucleotide Pathway

**b****c****d****e**

Supplemental Figure 1. Nucleotide-deficiency resistant large B cell lymphoma specifically upregulates dipeptides and their transporter SLC15A3.

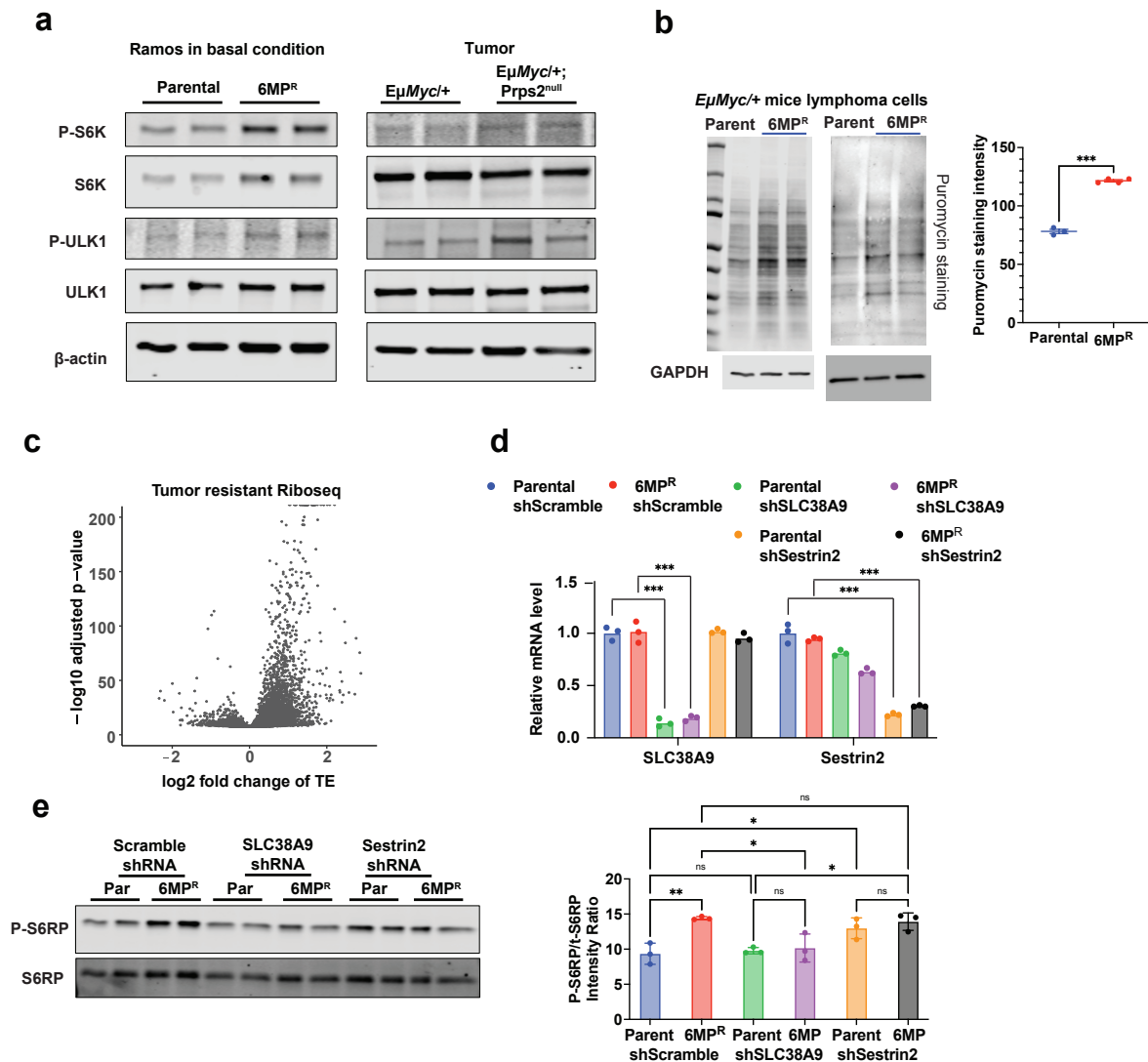
a. Volcano plot of metabolites comparing lymphoma from E μ -Myc/+; *Prps2*^{null} (Myc KO TD) mice to E μ -Myc/+ (Myc TD) mice in each sub-pathway within nucleotide pathway, and **b.** in each sub-pathway within peptide pathway. **c.** Two-dimensional plot of log₂ fold change in gene expression at the translational (RPF: ribosome-protected fragments, x-axis) and RNA (y-axis) levels in lymphomas from E μ -Myc/+; *Prps2*^{null} and E μ -Myc/+ mice. Red points indicate genes upregulated at both transcriptional and translational levels. **d.** Immunoblot analysis of indicated proteins in B cells from E μ -Myc/+ and E μ -Myc/+; *Prps2*^{null} mice. β -actin serves as the loading control. **e.** Volcano plot of metabolites comparing lymphoma from E μ -Myc/+; *Prps2*^{null} mice to E μ -Myc/+ mice in histidine metabolism sub-pathway.



Supplemental Figure 2. 6MP-resistant human lymphomas upregulate dipeptide uptake and SLC15A3.

a. Standard curve of AMCA reading in the lysates of cells incubated with different concentrations of AMCA labeled dipeptide. **b.** Relative mRNA levels of *SLC15A3* in parental and 6MP-resistant Ramos cells without or with shRNA-mediated knockdown of *SLC15A3*. **c.** Representative flow cytometric analysis of dipeptide-AMCA uptake in parental and 6MP-resistant Ramos without or with shRNA-mediated knockdown of *SLC15A3*. **d.** Gating strategies for flow cytometric analysis of dipeptide-AMCA in Ramos cells.

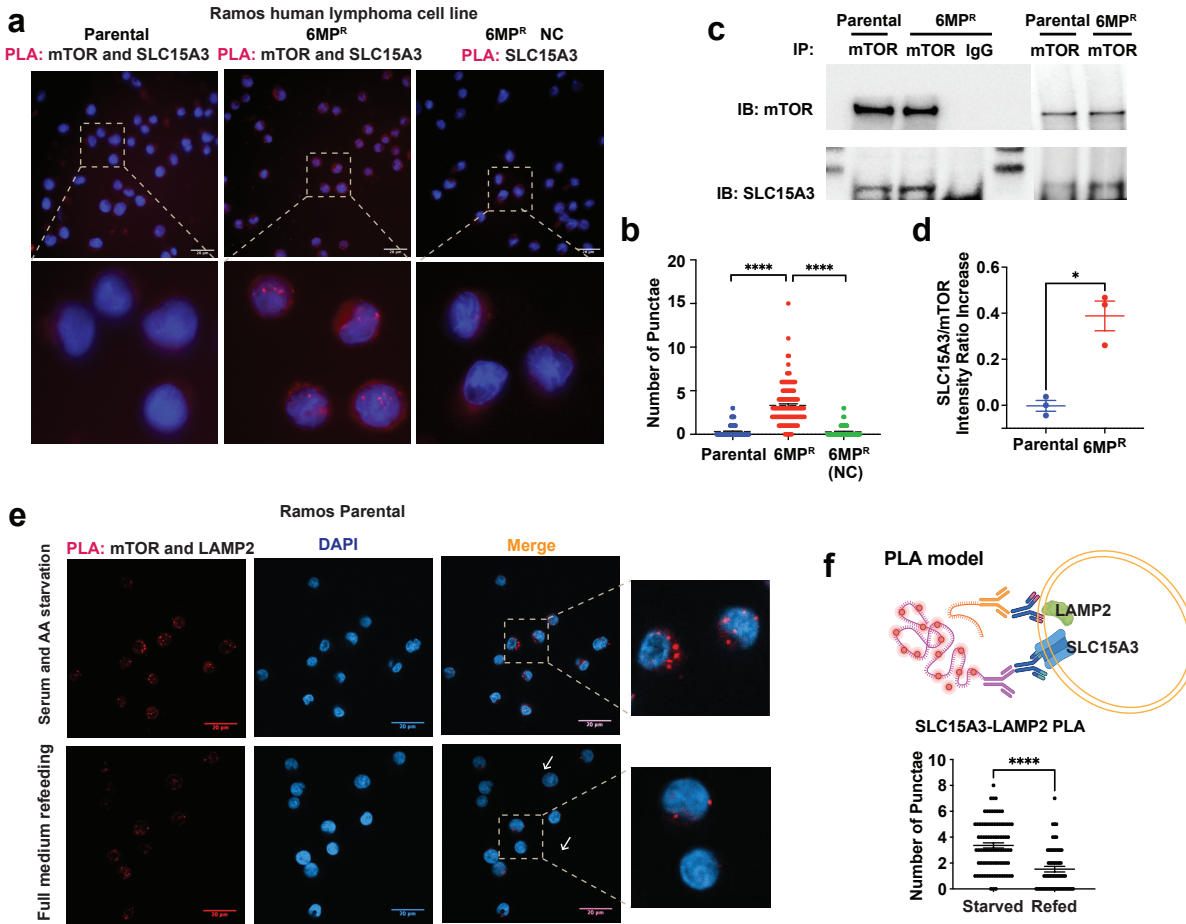
Individual data and mean $\pm$ SEM were shown in a and b, a was analyzed using simple linear regression, b was analyzed using 1-way ANOVA, ** $P < 0.01$, **** $P < 0.0001$.



Supplemental Figure 3. Elevated dipeptides are associated with enhanced mTORC1 activation in resistant lymphomas.

a. Immunoblot analysis of indicated proteins in parental or 6MP-resistant Ramos cells under full medium condition, and in Eμ-Myc/+; *Prps2*^{null} and Eμ-Myc/+ lymphomas. **b.** Immunoblot analysis and quantification of puromycin incorporation for 30 min in parental and 6MP-resistant Eμ-Myc/+ cells under basal condition, GAPDH serves as the loading control. **c.** Volcano plot of log₂ fold change of translation efficiency (TE) and -log₁₀ of p-value of translation efficiency of genes when comparing Eμ-Myc/+; *Prps2*^{null} and Eμ-Myc/+ lymphoma. **d.** Relative mRNA levels of *SLC38A9* and *SESTRIN2* in parental and 6MP-resistant Ramos cells without or with shRNA-mediated knockdown of *SLC38A9* or *SESTRIN2*. **e.** Immunoblot analysis of indicated proteins in parental and 6MP-resistant Ramos cells without or with shRNA-mediated knockdown of *SLC38A9* or *SESTRIN2*, and the quantification of P-S6RP/t-S6RP ratio.

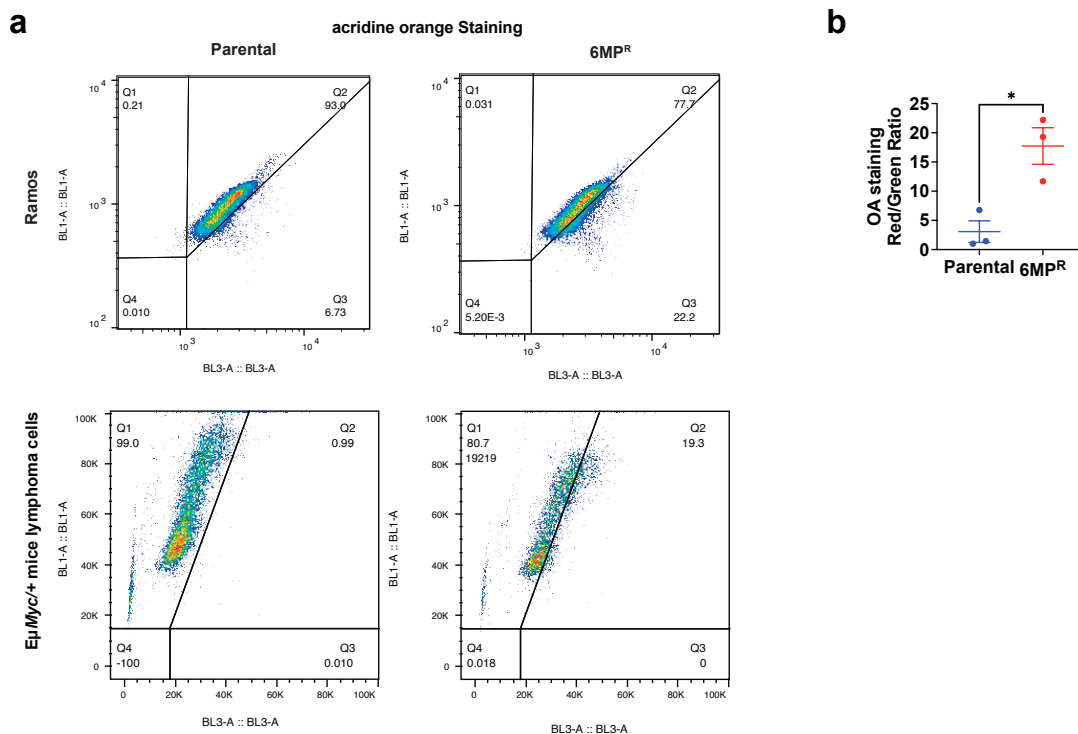
Individual data and mean ± SEM were shown in b, d, and e, and b was analyzed using t-test, d was analyzed using 2-way ANOVA, e was analyzed using 1-way ANOVA, ns P > 0.05, *P < 0.05, **P < 0.01; ***P < 0.001; ****P < 0.0001.



Supplemental Figure 4. SLC15A3 co-localizes with mTOR in 6MP-resistant lymphoma.

a. Representative images and **b.** quantification of proximity ligation assay (PLA) staining (red) using mTOR and SLC15A3 antibodies in parental and 6MP-resistant Ramos cells. PLA staining using only the SLC15A3 antibody in 6MP-resistant cells serves as a negative control. **c.** Immunoblot of co-IP using mTOR antibody or IgG in parental and 6MP-resistant Ramos cells. **d.** Relative increase of pull-down intensity ratio of SLC15A3 compared to mTOR in parental and 6MP-resistant Ramos cells. **e.** Representative images and **f.** quantification of proximity ligation assay (PLA) staining (red) using LAMP2 and SLC15A3 antibodies in Ramos cells after 45 min amino acid starvation and 15 min refeeding with full medium.

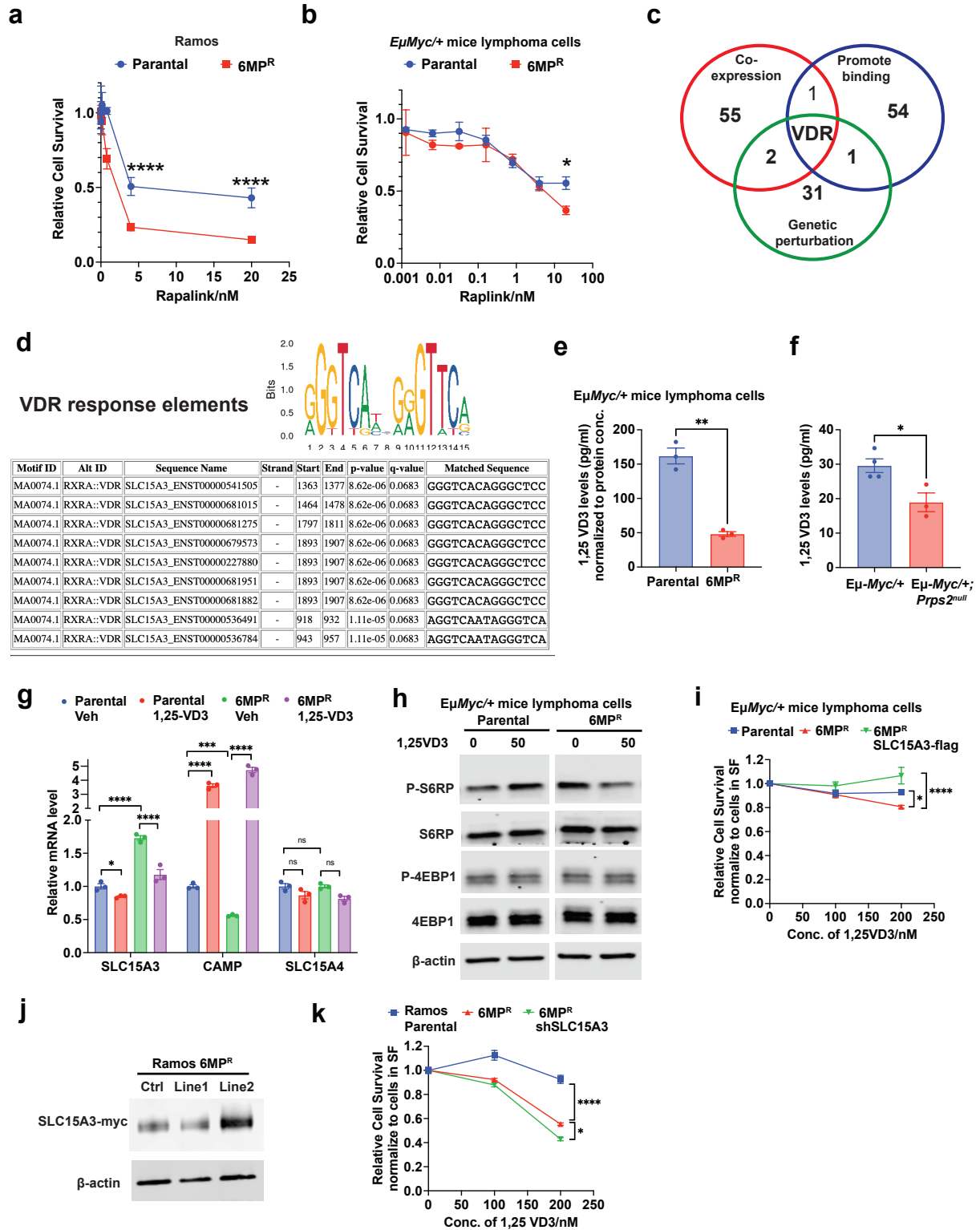
Individual data and mean $\pm$ SEM were shown in d and f were analyzed using t-test, b was analyzed using 1-way ANOVA, * $P < 0.05$, **** $P < 0.0001$.



Supplemental Figure 5. Enhanced lysosome acidification in 6MP-resistant cells.

a. Representative flow cytometric analysis of red and green fluorescence in Acridine Orange–stained cells, displayed using a red/green ratio–based thresholding strategy. **b.** Quantification of the red/green fluorescence ratio, corresponding to Q3 in the upper panel of **a** and Q2 in the lower panel of **a**.

Individual data and mean $\pm$ SEM were shown in **b**, and analyzed using t-test, * $P < 0.05$.



Supplemental Figure 6. Resistant lymphomas are more sensitive to rapamycin and active VD3 in vitro and in vivo.

a. Relative cell survival of parental and 6MP-resistant Ramos cells treated with different concentrations of Rapalink for 2 days. **b.** Relative cell survival of parental and 6MP-resistant E μ -Myc/+ lymphoma cells treated with different concentrations of Rapalink for 2 days. **c.** Venn diagram showing the overlap of genes identified by co-expression analysis, promoter binding, and genetic perturbation. **d.** VDR response element motif and significant matched sequences identified at the upstream of SLC15A3 promoter region. **e.** Cellular 1,25-VD3 levels normalized by protein levels in parental and 6MP-resistant E μ -Myc/+ lymphoma cells. **f.** Cellular 1,25-VD3 levels normalized by protein levels in E μ -Myc/+ or E μ -Myc/+;Prps2^{null} lymphomas. **g.** Relative mRNA levels of SLC15A3, SLC15A4 and VDR downstream target CAMP in parental (blue) and 6MP-resistant Ramos cells (red) treated with 100 nM 1,25-VD3 for 24h. **h.** Immunoblot analysis of indicated proteins in parental or 6MP-resistant E μ -Myc/+ lymphoma cells treated with 0 or 50 nM 1,25-VD3 for 24 h. **i.** Relative cell survival of parental and 6MP-resistant E μ -Myc/+ lymphoma cells without and with overexpression of SLC15A3 cDNA (line2) treated with different concentrations of 1,25-VD3 for 2 days. **j.** Immunoblot analysis of indicated proteins in 6MP-resistant Ramos cells infected without or with SLC15A3 cDNA lentivirus (line1, line2). **k.** Relative cell survival of parental and 6MP-resistant Ramos cells, with or without shSLC15A3, following treatment with different concentrations of 1,25-VD3 for 2 days. Individual data and mean $\pm$ SEM were shown in a, b, e, f, g, i and k, and e, e and f were analyzed using t-test, a, b, g, i and k were analyzed using 2-way ANOVA, *P < 0.05, **P < 0.01; ****P < 0.0001.